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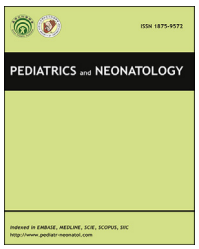

ORIGINAL ARTICLE

Risk Factors for Necrotizing Enterocolitis in Neonates: A Retrospective Case-Control Study

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Key Words
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Background: Necrotizing enterocolitis (NEC) in neonates is devastating, and risk-factor identification is crucial. This study aimed to evaluate risk factors for NEC in different gestational age (GA) groups.
Methods: Risk factors associated with NEC were investigated using a retrospective case-control design. Patients with Bell's Stage NEC $\geq$ II were divided into three groups based on GA: I, $<$34 weeks; II, $\geq$34 weeks but $<$37 weeks; III, $\geq$37 weeks. Each case was paired with two GA- and weight-matched controls. Data were collected from medical records, and univariate and conditional logistic regression analyses employed.
Results: A total of 238 cases and 476 controls were enrolled. Variation in the months when NEC was diagnosed was noted, with a peak in January and a trough in August. Intrahepatic cholestasis of pregnancy and transfusion with packed red blood cells were significantly associated with NEC in preterm infants. Meconium aspiration syndrome was an independent risk factor for a greater chance of NEC development in full-term infants. Postnatal asphyxia and sepsis were associated with an increased risk of NEC in all groups. Probiotic use was associated with a reduced risk of NEC. Patients aged $>$34 weeks with congenital heart disease were more likely than controls to have NEC.

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Conclusion: Intrahepatic cholestasis of pregnancy and meconium aspiration syndrome may be new risk factors for NEC.

1. Introduction

Necrotizing enterocolitis (NEC) is a common and devastating condition in neonates.[1] In the USA, the incidence of NEC is 0.7—1 per 1000 live births, with a significantly higher incidence among very low-birth-weight infants (5—10%).[2,3] In general, it is thought that 90% of all NEC cases are premature, but full-term infants account for approximately 7—25% of NEC cases.[3—5] In some cases, the incidence of NEC has increased because of ever-improving survival in the smallest infants.[6] A severe complication, neurodevelopmental delay, can develop in 25% of patients recovering from NEC resulting in catastrophic long-term consequences. NEC is associated with substantial medical burdens to both families and society.[7,8] The pathogenesis of NEC is incompletely understood, and epidemiological observations strongly suggest a multifactorial cause.[9,10]

As a consequence of the fulminant nature of NEC, it is unlikely that new diagnostic and treatment strategies will provide major breakthroughs in reducing the associated mortality and morbidity. The identification of possible risk factors is more likely to provide better results.[11,12] Numerous studies have been undertaken to investigate the risk factors for NEC. Commonly observed risk factors are prematurity, low birth-weight, enteral feeding, blood transfusion, and sepsis. Breast milk and probiotics have been demonstrated to be protective factors.[13—17] However, whether neonates with other risk factors might also have a chance of contacting NEC is not clear.

Shemer et al[18] reported previously that intrahepatic cholestasis of pregnancy (ICP) could increase the risk of preterm delivery and other adverse fetal outcomes. Nissen et al[19] reported that a family history of allergic disease may be associated with sensitization of the intestinal tract in neonates and children. Cayabyab et al[20] reported that meconium aspiration syndrome (MAS) may lead to fetal hypoxia via lung dysfunction and systemic inflammatory response. The above three clinical situations may be also associated with an increased risk of NEC. However, well-powered studies that can elucidate the association between the above conditions with NEC are lacking.

We aimed to evaluate the risk factors mentioned above and reconfirmed other, previously demonstrated risk factors. We also explored whether their effects differ by gestational age (GA) in a retrospective case-control study.

2. Materials and methods

2.1. Design and setting of our study

We undertook a retrospective case-control study to investigate a range of factors on the risk of NEC in different GA groups. The Chongqing Children's Hospital is a tertiary-care teaching hospital in the city of Chongqing, China. Our neonatal diagnostic center serves >6000 neonates every year.

Infants with NEC were identified by the International Classification of Diseases (10^{th} revision) codes. The Chongqing Children's Hospital used the ICD-10 system to replace ICD-9 from January 1, 2010. Clinical and demographic information of each patient was recorded in the electronic medical record system (MRS) at Chongqing Children's Hospital, and was anonymized before analyses. The study protocol was approved by the Institutional Review Board of Chongqing Children's Hospital.

2.2. Population selection

Data collection was conducted through a review of the patients' medical records. Patients were identified if they were classified in the database as having proven NEC (Bell Stage ≥ II) between March 1, 2010 and March 1, 2015.[21,22] Two controls were selected from the database for each patient and matched for birthdate (difference of <2 months), estimated GA (<1 week apart), and birth-weight (difference of <100 g).[23] Control patients were those admitted to the same institution for medical reasons other than NEC before hospital discharge. We stratified infants by GA into three groups: I, <34 weeks; II, ≥34 weeks but <37 weeks; III, ≥37 weeks. Exclusion criteria were any immunodeficiency disease, inherited metabolic disorder, major gastrointestinal malformations, as well as patients for whom eligible controls could not be found or for whom relevant information was insufficient, and those who had undergone abdominal surgery before enrollment. Medical records for each case and control were reviewed independently by two investigators who were unaware of the study aims. Disagreements were resolved by consensus between the two investigators.

2.3. Study protocol and data collection

Information on risk factors was obtained from the electronic MRS of Chongqing Children's Hospital. Data was collected until the end of admission for both NEC and non-NEC groups. Key risk factors were ICP, family history of allergy, MAS, congenital heart disease (CHD),[24,25] postnatal asphyxia,[26] oxygen inhalation (number of days), sepsis, and transfusion of packed red blood cells (PRBCs). Other characteristics of patients were birthdate, GA, birth-weight, sex, prenatal/maternal data, mode of delivery, and place of birth. We also reported exposure to breast milk and probiotics to ascertain if they may be associated with a reduced risk of NEC.

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2.4. Statistical analyses

Descriptive assessments of patients were conducted to examine the variables of interest. Data are the mean ± standard deviation for continuous variables, and absolute frequencies or percentages for categorical variables. McNemar's test and Fisher's exact test were used for categorical variables. For continuous variables, groups were evaluated using the Student *t*-test and Mann—Whitney *U* test.

Conditional logistic regression analyses were performed to determine the possible risk factors for NEC after inclusion in the model of significant variables identified in univariate analyses. Odds ratios were estimated with confidence intervals at 95%. A p value < 0.05 was considered significant. All analyses were carried out using SPSS v19.0 (IBM, Armonk, NY, USA).

3. Results

We identified 238 cases (Grade-II NEC = 190; Grade-III NEC = 48) and 476 controls. Figure 1 illustrates the selection process for these patients.

Figure 2 shows the seasonal variation in the months when babies with NEC were born, with a peak in January and a trough in August, which persisted after subgroup analyses for GA groups.

Table 1 shows the demographic characteristics and results with risk factors in univariate analyses among different groups. Table 2 presents the results of conditional logistic regression analyses including all parameters with $p < 0.05$ in univariate analyses.

In the final model, the variables significantly associated with NEC in Group I were ICP, postnatal asphyxia, sepsis, and transfusion of PRBCs. Probiotics were associated with a reduced risk of NEC. When compared with Group I, Group II showed similar results after conditional logistic regression analyses: ICP, postnatal asphyxia, sepsis, transfusion of PRBCs and probiotics. Moreover, patients aged 34—37 weeks were more likely than matched controls to have CHD. In Group III, postnatal asphyxia, MAS, sepsis, and CHD were independent risk factors for a greater chance of developing NEC compared with controls.

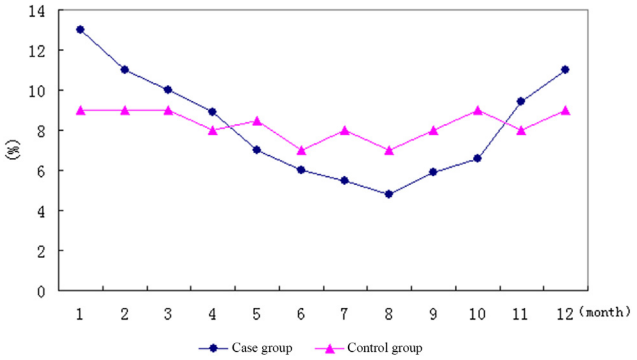

Figure 2 Seasonal variation in the months when babies with NEC are born. NEC = necrotizing enterocolitis.

4. Discussion

In this case-control study, seasonal variation in the prevalence of NEC was observed in a population in southwest China. Specific conditions were associated with an increased risk of NEC: ICP, postnatal asphyxia, MAS, sepsis, CHD, probiotic use, and transfusion of PRBCs. Relative effects of many of these risk factors differed in different GA

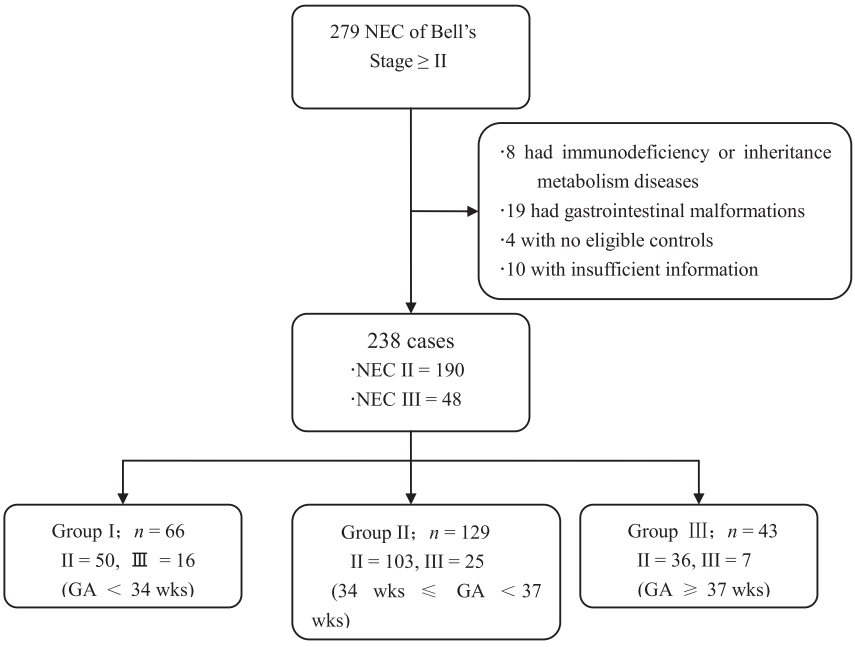

Figure 1 Identification and inclusion of patients in our study. GA = gestational age; NEC = necrotizing enterocolitis.

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Table 1 Demographic characteristics and clinical variables of interest of cases and controls.

	Group I (GA < 34 wks)			Group II (34 wks ≤ GA < 37 wks)			Group III (GA ≥ 37 wks)		
	Cases (n = 66)	Controls (n = 132)	p	Cases (n = 129)	Controls (n = 258)	p	Cases (n = 43)	Controls (n = 86)	p
Demographic characteristics									
Gestational age (wk)	29.8 ± 1.6	30.7 ± 1.5	0.142	34.8 ± 0.7	35.6 ± 1.2	0.224	38.9 ± 1.2	39.4 ± 2.1	0.186
Birth-weight (g)	1595 ± 368	1680 ± 262	0.551	2360 ± 214	2440 ± 198	0.635	2886 ± 323	2940 ± 176	0.467
Maternal age	28.4 ± 2.1	27.3 ± 3.0	0.323	27.8 ± 1.7	27.1 ± 2.4	0.247	28.6 ± 0.8	29.1 ± 1.6	0.276
Gender									
Male	38 (57.6)	75 (56.8)	0.381	76 (58.9)	142 (55.0)	0.292	26 (60.5)	50 (58.1)	0.306
Female	28 (42.4)	57 (43.2)	0.438	53 (41.1)	116 (45.0)	0.203	17 (39.5)	36 (41.9)	0.319
Mode of delivery									
Vaginal	26 (39.4)	58 (43.9)	0.474	54 (41.9)	106 (41.1)	0.469	19 (44.2)	39 (45.3)	0.397
C-section	40 (60.1)	74 (56.1)	0.476	75 (58.1)	152 (58.9)	0.394	24 (55.8)	47 (54.7)	0.308
Place of birth									
Urban	32 (48.5)	68 (51.5)	0.287	58 (45.0)	108 (41.9)	0.201	21 (48.8)	40 (46.5)	0.103
Rural	28 (42.4)	54 (40.9)	0.108	60 (46.5)	115 (44.6)	0.115	17 (39.6)	38 (44.2)	0.108
Unknown	6 (9.1)	10 (7.6)	0.311	11 (8.5)	35 (13.5)	0.295	5 (11.6)	8 (9.3)	0.302
Risk factors									
ICP	9 (13.6)	8 (6.6)	**0.021**	21 (16.3)	18 (6.9)	**0.012**	3 (6.9)	4 (4.7)	0.209
Asphyxia after birth	30 (45.5)	34 (25.8)	**0.011**	48 (37.2)	46 (17.8)	**0.019**	23 (53.5)	21 (24.4)	**0.013**
Family allergy history	6 (9.1)	9 (6.8)	0.198	11 (8.5)	21 (8.1)	0.204	4 (9.3)	7 (8.1)	0.387
Breast milk	15 (22.7)	28 (21.2)	0.418	32 (24.9)	70 (27.1)	0.209	11 (25.6)	24 (27.9)	0.307
MAS	1 (1.5)	3 (2.2)	0.682	5 (3.8)	6 (2.4)	0.054	5 (11.6)	5 (5.8)	**0.015**
Sepsis	28 (42.4)	23 (17.4)	**0.006**	48 (38.1)	41 (15.9)	**0.011**	11 (25.6)	12 (13.9)	**0.021**
Oxygen (d)	5.8 ± 1.1	4.6 ± 0.9	0.114	5.5 ± 1.6	5.1 ± 1.3	0.175	4.8 ± 0.7	4.3 ± 1.2	0.248
CHD	13 (19.7)	25 (18.9)	0.431	21 (16.3)	25 (9.7)	**0.016**	7 (16.3)	5 (5.8)	**0.024**
Probiotics	4 (6.1)	15 (11.4)	**0.012**	9 (7.0)	34 (13.2)	**0.021**	3 (6.9)	7 (8.1)	**0.181**
Transfusion of PRBC	16 (24.2)	10 (7.5)	**0.011**	26 (20.1)	18 (7.0)	**0.017**	2 (4.6)	3 (3.5)	0.013

A p-value < 0.05 was considered to be statistically significant. Values are expressed as mean ± SD or n (%).
CHD = congenital heart disease; ICP = intrahepatic cholestasis of pregnancy; MAS = meconium aspiration syndrome; PRBC = packed red blood cells.

groups. By exploring possible risk factors, new strategies could be provided to reduce the risk of NEC among these groups.

Seasonal variation of the months in which babies with NEC were born was broadly in accordance with that seen in other studies. Snyder et al[27] reported a period of 6 months with peaks in June and December. Ahle et al[28] reported a period of 12 months with a peak in November and a trough in May. In contrast, our data demonstrated a period of 12 months with a peak in January and a trough in August. A peak during cold months may indicate that climatic and environmental factors influence the occurrence of NEC. Also, the period when NEC occurs was identical after subgroup analyses for different GA groups. However, our conclusion might be biased as calculations were restricted to premature infants in hospital, which may not reveal the true incidence. In fact, a large and truly population-based research in Chongqing will soon be initiated by our institution to obtain more reliable data.

ICP was associated with an increased risk of NEC for preterm infants, which has been reported only rarely in the literature. Although the cause of ICP is complex and largely unclear, several studies have consistently found an association between ICP and adverse outcome in fetuses, including spontaneous preterm birth, asphyxial events, as well as meconium staining of amniotic fluid, placenta and membranes,[18,29] Costoya et al[30] reported that increased levels of bile acids in serum cause a reduction in size of intervillous spaces as a result of swelling of trophoblasts and edema of the villous stroma within the placenta. These changes could lower oxygenation to the fetus by reducing maternal blood flow to intervillous spaces and lead to hypoxia-ischemia events, which is probably related to relative production of vascular regulators and contributes substantially to NEC pathogenesis. This hypothesis must be tested in a prospective study.

MAS is a major cause of respiratory failure and a common diagnosis in full-term infants. Increased levels of neutrophils and proinflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-α also have important roles in the pathogenesis of MAS.[20,31] However, in our study, a large amount of cytokines produced in the lung may have induced inflammation and altered the protective barriers in the intestine *via* the vascular circulation. The intestinal tracts of neonates exposed to meconium-stained amniotic fluid may also lead to an exaggerated immune response in the intestine, similar to the way that MAS-associated lung injury influences the environmental conditions of the intestinal tract, thereby increasing the risk of NEC. In Group III, all the patients with MAS had nonsevere disease, and only five (50%) become symptomatic, requiring

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Table 2 Conditional logistic regression analyses of the risk factors associated with NEC.

		b	S_b	*p*	Wald $\chi 2$	OR	95% CI
Group I	ICP	0.741	0.187	**0.036**	8.327	2.17	(1.69, 5.61)
	Asphyxia after birth	0.941	0.036	**0.008**	2.187	2.51	(1.61, 4.47)
	Sepsis	1.461	0.021	**0.003**	0.285	3.95	(1.92, 6.38)
	Probiotics	−0.119	0.216	**0.036**	0.998	0.87	(0.38, 1.17)
	Transfusion of PRBC	1.339	0.018	**0.012**	2.112	3.02	(1.36, 6.02)
Group II	ICP	0.764	0.221	**0.019**	7.379	2.66	(1.59, 6.24)
	Asphyxia after birth	0.717	0.028	**0.018**	1.723	1.47	(1.31, 4.16)
	Sepsis	1.378	0.045	**0.009**	0.339	3.85	(1.72, 7.64)
	Probiotics	−0.145	0.376	**0.025**	0.719	0.79	(0.51, 0.93)
	CHD	0.712	0.032	**0.021**	2.891	1.61	(1.01, 4.23)
	Transfusion of PRBC	1.468	0.026	**0.005**	0.894	2.85	(1.42, 6.31)
Group III	Asphyxia after birth	0.651	0.017	**0.031**	2.496	3.15	(1.67, 4.78)
	MAS	0.872	0.036	**0.014**	0.632	1.49	(0.93, 3.82)
	Sepsis	1.361	0.019	**0.011**	0.756	3.41	(1.14, 5.79)
	Probiotics	−0.223	0.186	**0.019**	0.667	0.92	(0.41, 1.54)
	CHD	0.696	0.028	**0.011**	1.893	2.54	(1.15, 3.71)

Values are expressed as mean ± SD or *n* (%).
CHD = congenital heart disease; CI = confidence interval; ICP = intrahepatic cholestasis of pregnancy; MAS = meconium aspiration syndrome; OR = odds ratio; PRBC = packed red blood cells.

oxygen supplementation rather than ventilatory support. In our study, the regression coefficient was 0.872 for MAS and 0.651 for postnatal asphyxia. Therefore, MAS may be an independent contributor to the development of NEC. To clarify this hypothesis, large controlled prospective trials are needed.

Several studies have suggested that CHD is associated with an increased risk of NEC among the late-preterm and full-term infants.[25] After adjustment for GA, the association was obvious for late-preterm and full-term infants, again suggesting that CHD adds to the overall risk of NEC in this population. Studies have suggested that reduced mesenteric perfusion is the main cause, although other etiologic factors also contribute to the development of NEC.[32] The finding that the risk for NEC is correlated with asphyxia also strongly supports the notion that insufficiency in mesenteric flow is a risk factor for NEC development.[26]

Our data supports the results of studies which have described an association between NEC and PRBC transfusions.[33] A recent retrospective study reported that 27% of infants are given PRBC transfusions within 48 hours of the diagnosis of NEC.[34] Anemia of prematurity and hyperbilirubinemia are common in preterm infants, and PRBC transfusion has an important role in therapy.[35] We also included breast milk as a risk factor, but it did not reach significance in conditional logistic regression analyses. In our experience, this phenomenon results from the low proportion of breast feeding in Chinese society.[36] In addition, the association of NEC with sepsis and probiotics was in accordance with other studies.[13,17]

Our study had specific strengths. This was the first study to report the risk factors for NEC in which different GA groups were included simultaneously. Also, it comprised a large sample population investigated over 5 years, thereby providing high statistical power for the detection of new potential risk factors. Among these risk factors, ICP and MAS have been rarely reported.

However, our study had limitations because of its retrospective nature of data collection. First, our study may have been subject to various sources of bias. In general, a multicenter and large-sample study can diminish any bias in the prevalence of admission, but our study was based at one center. Second, due to the limited number of patients graded as ≥ II using the Bell classification, some mild cases of NEC may have been missed, thereby leading to underestimation of associations. Finally, the retrospective nature of our study limited us to information recorded in the MRS, and some important data may not have been recorded.

5. Conclusion

A range of conditions was found to be associated with an increased risk of NEC. In this study, ICP and MAS may be new risk factors for NEC. Attention should be paid to these risk factors in future medical practices. However, the results of our study must be interpreted with caution due to the limitations imposed by the study design. To validate these findings, a larger sample and multicenter prospective study are needed. If these factors are validated, appropriate medical interventions to reduce the devastating impact of this disease can be implemented.

Conflicts of interest

None declared.

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